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Original Article

USP3 Promotes Glioma Progression by Stabilizing PLK1 through Deubiquitination

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ABSTRACT: Glioma is an aggressive primary brain tumor with high morbidity and mortality, and it requires novel therapeutic targets. Ubiquitination and deubiquitination pathways regulate tumor progression. USP3, a deubiquitinating enzyme, and PLK1, a cell cycle kinase, have been linked to cancer cell proliferation. This study examined whether USP3 modulates PLK1 through deubiquitination to influence glioma progression. USP3 expression in glioma versus normal tissues was analyzed using TCGA datasets. Gain-of-function and loss-of-function models were generated by USP3 overexpression in U-251MG cells and USP3 knockdown in DK-MG cells. Proliferation was assessed by CCK-8, cell cycle distribution was analyzed by flow cytometry, and migration and invasion were evaluated using Transwell assays. Nude mouse xenografts were used to assess tumor growth, and PCNA immunohistochemistry was performed. Co-immunoprecipitation and Western blotting were used to validate the USP3–PLK1 interaction and to assess PLK1 ubiquitination. USP3 was upregulated in glioma tissues. USP3 overexpression enhanced U-251MG proliferation, promoted cell cycle progression, and increased invasion, while USP3 knockdown suppressed these phenotypes in DK-MG cells. Xenografts derived from USP3-overexpressing cells grew faster and showed higher PCNA levels, whereas USP3 knockdown reduced tumor growth and PCNA staining. Mechanistically, USP3 bound to PLK1, reduced K48-linked ubiquitination of PLK1, and stabilized PLK1 protein levels. USP3 knockdown increased PLK1 ubiquitination and reduced PLK1 abundance. Overall, USP3 promoted glioma malignancy by deubiquitinating and stabilizing PLK1, which enhanced proliferation and invasion. Targeting the USP3–PLK1 axis may represent a potential therapeutic strategy for glioma.

Keywords: USP3, PLK1, glioma, deubioquitination, proliferation

INTRODUCTION

Glioma is a common and challenging primary tumor of the central nervous system. It is characterized by high invasiveness and frequent recurrence, which pose a serious threat to human health. Globally, the incidence of glioma continues to increase [1]. Prognosis remains poor across pathological grades, and the median survival of patients with high-grade glioma is only 12 to 15 months [2]. Current comprehensive treatments include surgery, radiotherapy, and chemotherapy. Although these approaches extend survival to some extent, glioma remains difficult to cure because its pathogenesis is not fully understood. In addition, existing therapies often cause substantial adverse effects, reduce quality of life, and fail to prevent recurrence. Therefore, new therapeutic targets are urgently needed [3].

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Protein ubiquitination and deubiquitination are critical regulatory processes in cells. They regulate key physiological functions, including cell cycle control, signal transduction, and DNA damage repair [4]. During tumor development, disruption of this regulatory balance leads to abnormal expression and function of cancer-related proteins [5]. USP3 is an important member of the deubiquitinating enzyme family [6]. Aberrant USP3 expression has been reported in multiple solid tumors, including breast cancer and lung cancer [7]. By deubiquitinating substrate proteins, USP3 regulates malignant behaviors such as proliferation, resistance to apoptosis, and metastasis [8]. However, the role of USP3 in glioma remains incompletely defined. Limited studies suggest that USP3 expression correlates with tumor malignancy, but its effects on specific cellular processes and the downstream pathways involved remain unclear and require further investigation.

PLK1 is a key regulator of the cell cycle and is essential for mitosis [9]. Spindle assembly, chromosome alignment, and cytokinesis depend on precise PLK1 control [10]. PLK1 overexpression is common in tumor cells, where it accelerates cell cycle progression, promotes proliferation, and associates with invasiveness and poor prognosis [11]. In many tumor models, PLK1 inhibition suppresses tumor cell growth [12]. However, in glioma, the interaction network between PLK1 and other key regulators remains insufficiently characterized. In addition, strategies to target PLK1 effectively while limiting injury to normal cells remain an important unresolved challenge.

This study investigates the deubiquitination-mediated regulation of PLK1 by USP3. This question is relevant to both mechanistic understanding of glioma pathogenesis and the development of new therapeutic strategies. The findings are expected to support improved approaches to glioma diagnosis and treatment by clarifying a previously underexplored regulatory axis. They may also provide a rationale for future precision therapies and treatment optimization.

MATERIALS AND METHODS

Glioma cell lines

The human glioma cell lines U-251MG and DK-MG were obtained from ATCC. Cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin in a humidified incubator at 37°C with 5% CO2. Cells were passaged every 2 to 3 days. Cell line identity was confirmed by short tandem repeat profiling with concordance to reference profiles and no evidence of cross-contamination. Mycoplasma status was assessed with a commercial detection kit, and results were negative.

Cell transfection and lentiviral infection

USP3 overexpression by plasmid transfection A pCDH-CMV-MCS-EF1-copGFP construct containing the USP3 coding sequence was prepared. U-251MG cells were seeded at 6×10^5 cells per well in 6-well plates. Transfection was performed at 70% to 80% confluence according to the Lipofectamine 2000 protocol. Plasmid DNA (2 μg) was diluted in 250 μL Opti-MEM. Lipofectamine 2000 (5 μL) was diluted in 250 μL Opti-MEM. Dilutions were incubated for 5 min at room temperature, combined, and incubated for 20 min to form complexes. Complexes were added to cells. Medium was replaced with complete medium after 6 h. GFP fluorescence was assessed at 48 h, and cells were collected for downstream assays.

USP3 knockdown by lentiviral infection

Lentiviral vectors encoding USP3-targeting shRNA with a puromycin resistance cassette and matched negative control vectors were generated by Gemma Gene Company. DK-MG cells were seeded at 6×10^5 cells per well in 6-well plates. Infection was performed at 50% to 60% confluence. Lentivirus was thawed in a 37°C water bath and added at an MOI of 50 with 8 μg/mL polybrene. Medium was replaced with complete medium after 12 h. Puromycin selection (2 μg/mL) was performed for 7 to 10 days starting at 48 h to establish stable knockdown lines. Knockdown was verified by Western blot.

Immunoprecipitation and Western blot

Cell or tissue samples were lysed on ice in RIPA buffer supplemented with 1 mM PMSF for 30 min with vortexing for 10 s every 5 min. Lysates were centrifuged at 4°C at 12,000 rpm for 15 min, and supernatants were collected. Protein concentration was measured by BCA assay. Protein (40 μg) was mixed with loading buffer and denatured at 100°C for 5 min. Proteins were separated on 10% SDS-PAGE gels and transferred to PVDF membranes by wet transfer at 200 mA for 90 min. Membranes were blocked in 5% skim milk for 1 h at room temperature and incubated overnight at 4°C with primary antibodies against USP3 (Proteintech, 12490-1-AP), PLK1 (Proteintech, 10305-1-AP), ubiquitination, and GAPDH (Proteintech, 60004-1-Ig). Membranes were washed three times in TBST for 10 min per wash, incubated with appropriate secondary antibodies (1:5,000) for 1 h at room temperature, and washed three

additional times in TBST. Bands were detected by chemiluminescence and quantified in ImageJ.

RNA extraction and PCR-based analysis

Total RNA was extracted from cells or tissues using Trizol. Chloroform phase separation was performed, followed by isopropanol precipitation and ethanol washing. RNA was dissolved in RNase-free water and quantified. cDNA was generated by reverse transcription with RNA, reverse transcription primers, and reverse transcriptase using standard annealing, extension, and enzyme inactivation steps. PCR amplification was performed with cDNA template, gene-specific primers, and Taq DNA polymerase using 25 to 40 cycles of denaturation, annealing, and extension, followed by a final extension. PCR products were assessed by downstream detection methods, including sequencing.

Immunohistochemistry

Glioma surgical specimens were fixed in 10% formalin for more than 24 h, dehydrated, cleared, and paraffin embedded. Sections (5 μm) were prepared, baked at 65°C for 45 min, deparaffinized in xylene, and rehydrated through graded ethanol. Antigen retrieval was performed in citrate buffer (pH 6.0) using a pressure cooker. After steam release began, heating was maintained for 5 min, followed by natural cooling. Sections were washed in PBS. Endogenous peroxidase was blocked with 0.3% hydrogen peroxide and 1.5% horse serum for 10 min, followed by PBS washes. Sections were incubated overnight at 4°C with primary antibodies against USP3, PLK1, and PCNA (Proteintech, 10205-2-AP). After PBS washes, sections were incubated with universal rabbit or mouse IgG secondary antibody (1:200) for 1 h at room temperature. Signal was developed with the Ultraview DAB kit. Nuclei were counterstained with hematoxylin. Sections were dehydrated through graded ethanol, cleared in xylene, and mounted with neutral gum. Images were acquired on a Zeiss microscope at 200× magnification.

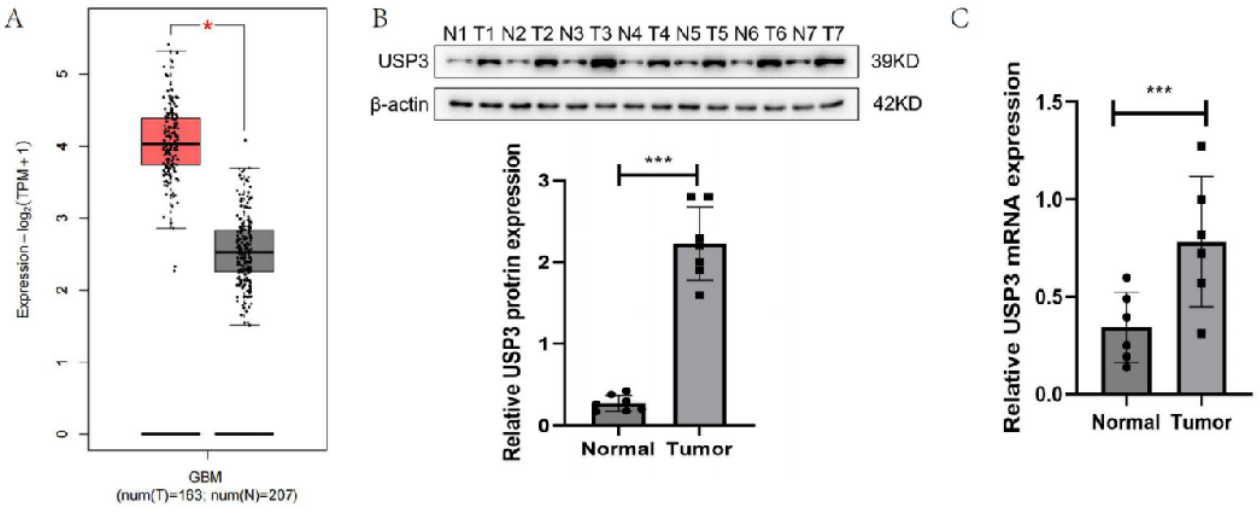

Figure 1. USP3 is upregulated in glioma. (A) TCGA analysis shows higher USP3 expression in glioma than in normal brain tissue. **(B)** Western blot analysis confirms elevated USP3 protein levels in glioma tissues. **(C)** RT-qPCR confirms increased USP3 mRNA levels in glioma tissues (Mann–Whitney U test). *P < 0.05, **P < 0.01, ***P < 0.001.

Cell proliferation assay (CCK-8)

At 48 h after transfection or infection, cells were seeded at 2.0 × 10^3 cells per well in 96-well plates with six replicate wells per group. Cells were incubated at 37°C with 5% CO2 and assessed at 24 h, 48 h, 72 h, and 96 h. At each time point, 10 μL CCK-8 reagent was added at a 1:10 reagent-to-medium ratio, followed by incubation for 2 h. Absorbance was measured at 450 nm using a microplate reader. Experiments were repeated three times.

Colony formation assay

Transfected or infected U-251MG and DK-MG cells were dissociated with trypsin and prepared as single-cell suspensions. Cells were seeded at 500 cells per well in 6-well plates with three replicate wells per group. Plates were incubated at 37°C with 5% CO2 for 10 to 14 days, and complete medium was replaced every 3 to 4 days. Wells were washed twice with PBS, fixed in 4% paraformaldehyde for 20 min, washed twice with PBS, and stained with crystal violet for 30 min at room

temperature. Plates were washed with water and air dried. Colonies with at least 50 cells were counted under a microscope. Colony formation rate was calculated as (number of colonies divided by number of seeded cells) × 100%. Experiments were repeated three times.

Cell invasion assay (Transwell)

The upper chamber was coated with 50 µL Matrigel diluted 1:8 in serum-free medium and incubated at 37°C for 4 to 6 h to allow gelation. Treated cells were resuspended at 2 × 10^5 cells/mL, and 100 µL cell suspension (2 × 10^4 cells) was added to the upper chamber. Medium with 10% fetal bovine serum was added to the lower chamber. Inserts were incubated at 37°C for 48 h. Post-incubation processing was performed as in the migration assay workflow. Experiments were repeated three times.

Wound healing assay

Transfected or infected U-251MG and DK-MG cells were seeded at 3 × 10^5 cells per well in 6-well plates and grown to full confluence. A straight vertical scratch was generated with a sterile 10 µL pipette tip. Wells were washed three times with PBS to remove debris, and serum-free medium was added. Images were captured at 0 h and 24 h in the same fields using an inverted microscope. Wound width was measured in ImageJ. Migration rate was calculated as (width at 0 h minus width at 24 h) divided by width at 0 h × 100%. Three replicate wells per group were included. Experiments were repeated three times.

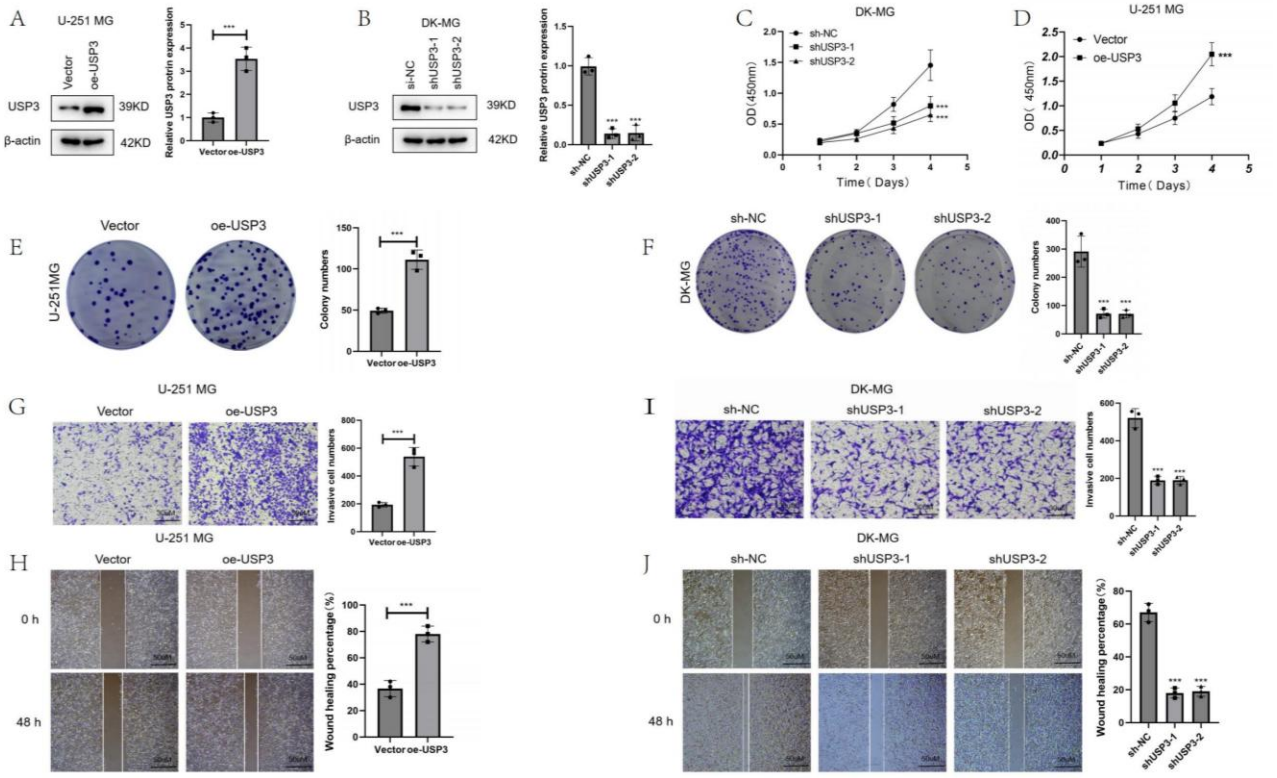

Figure 2. USP3 promotes glioma cell proliferation and invasion. (A, B) Western blot analysis was used to measure USP3 expression in U-251MG and DK-MG cells transfected with a USP3 overexpression plasmid or USP3 shRNA. **(C, D)** CCK-8 assays were performed in U-251MG and DK-MG cells transfected with a USP3 overexpression plasmid or USP3 shRNA. **(E, F)** Colony formation assays were conducted in U-251MG and DK-MG cells transfected with a USP3 overexpression plasmid or USP3 shRNA. **(G, H)** Transwell invasion assays were performed to evaluate invasive capacity in U-251MG and DK-MG cells transfected with a USP3 overexpression plasmid or USP3 shRNA. **(I, J)** Wound healing assays were used to assess migratory capacity in U-251MG and DK-MG cells transfected with a USP3 overexpression plasmid or USP3 shRNA (Mann–Whitney U test). *P < 0.05, **P < 0.01, ***P < 0.001.

Animal experiments

Male BALB/c nude mice (6 weeks old, 20 to 22 g) were acclimated for 1 week. U-251MG cells (USP3 overexpression and control) or DK-MG cells (USP3 knockdown and control) were prepared at 2 × 10^6 cells in 0.1 mL of a 1:1 mixture of Matrigel and DMEM. Cell suspensions were injected subcutaneously into both dorsal

flanks. Mice were monitored daily. When tumors reached approximately 2 mm in diameter, tumor length (L) and width (W) were measured with digital calipers, and volume was calculated as $0.52 \times L \times W^2$ for 10 days. In the overexpression cohort, intragastric dosing was performed with normal saline or RO-3306 (4 mg/kg) every 2 days. On day 10, mice were anesthetized with pentobarbital sodium overdose and euthanized by cervical dislocation. Tumors were excised, and tumor weight and volume were recorded. A subset of tumors was fixed in 10% paraformaldehyde for immunohistochemistry of USP3, PLK1, and Ki-67. All animal procedures were approved by the Animal Ethics Committee of Xuanwu Hospital, Capital Medical University (LYS[2021]118).

Statistical analysis

Statistical analyses were performed in GraphPad Prism 9.0. Replicates described in this study were treated as biological replicates. Normality was assessed with the Shapiro-Wilk test. Homogeneity of variances was assessed with Levene's test. Two-group comparisons were performed with Student's t-test for normally distributed data with equal variances, or with the Mann-Whitney U test otherwise. Repeated measures were analyzed by two-way repeated measures ANOVA with Bonferroni post hoc testing. Statistical significance was defined as $P < 0.05$.

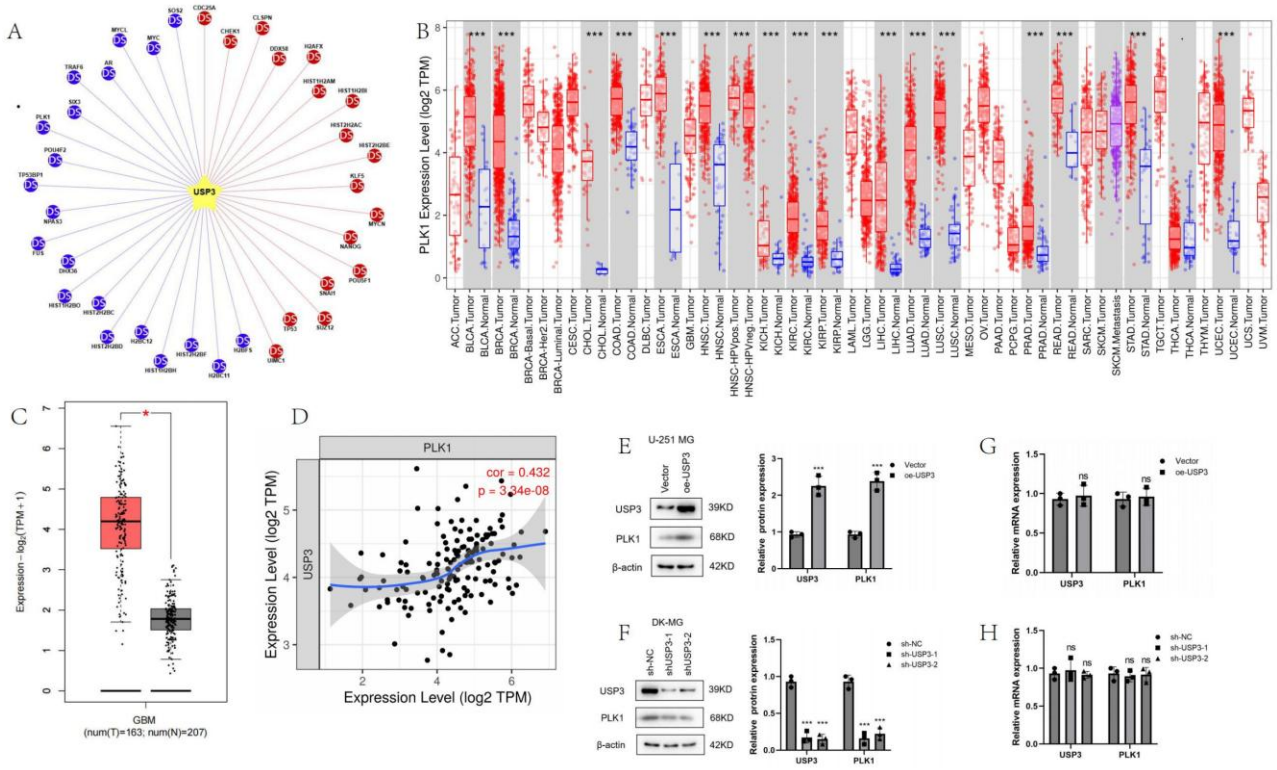

Figure 3. USP3 regulates PLK1 expression. (A) UbiBrowser analysis predicted USP3-interacting proteins and suggested an interaction between USP3 and PLK1. **(B)** TIMER analysis showed that PLK1 is upregulated in multiple tumor types. **(C)** GEPIA2 analysis indicated that PLK1 expression is higher in glioma than in normal brain tissue. **(D)** TIMER analysis showed a positive correlation between USP3 and PLK1 expression. **(E)** Western blot analysis of PLK1 protein levels in U-251MG cells transfected with a USP3 overexpression plasmid. **(F)** Western blot analysis of PLK1 protein levels in DK-MG cells transfected with USP3 shRNA. **(G)** RT-qPCR analysis of PLK1 mRNA levels in U-251MG cells transfected with a USP3 overexpression plasmid. (H) RT-qPCR analysis of PLK1 mRNA levels in DK-MG cells transfected with USP3 shRNA (Mann–Whitney U test). *P < 0.05, **P < 0.01, ***P < 0.001.

RESULTS

Upregulated USP3 expression in glioma

To evaluate USP3 expression in glioma, glioma-related gene expression datasets were obtained from The Cancer Genome Atlas (TCGA). Differential expression analysis identified USP3 as significantly dysregulated in glioma. USP3 expression was higher in glioma tissues than in normal brain tissues (Fig. 1A). To validate these findings, USP3 protein levels were assessed in clinical specimens by Western blot, which confirmed higher USP3 expression in glioma (Fig. 1B). In parallel, RT-qPCR analysis showed higher USP3 mRNA levels in glioma tissues than in normal tissues (Fig. 1C). Together, TCGA analysis and clinical validation supported elevated USP3

expression in glioma and supported follow-up functional studies.

USP3 promotes glioma cell proliferation and invasion Gain-of-function and loss-of-function studies were performed to define the role of USP3 in glioma cells. USP3 was overexpressed in U-251MG cells, and USP3 was silenced in DK-MG cells using lentiviral shRNA. USP3 modulation was confirmed by Western blot and RT-qPCR (Fig. 2A and Fig. 2B). In colony formation assays, USP3-overexpressing U-251MG cells formed more colonies than controls (Fig. 2E). In contrast, USP3-silenced DK-MG cells formed fewer colonies than controls (Fig. 2F). Cell proliferation was assessed by CCK-8 at 24 h, 48 h, 72 h, and 96 h. USP3 overexpression increased proliferation signals in U-251MG cells (Fig. 2C), while USP3 silencing reduced proliferation signals in DK-MG cells (Fig. 2D). Migration and invasion were assessed using wound healing and Transwell invasion assays. USP3 overexpression increased scratch closure at 48 h (Fig. 2G) and increased the number of invaded cells (Fig. 2I). USP3 silencing reduced scratch closure and reduced the number of invaded cells (Fig. 2H and Fig. 2J).

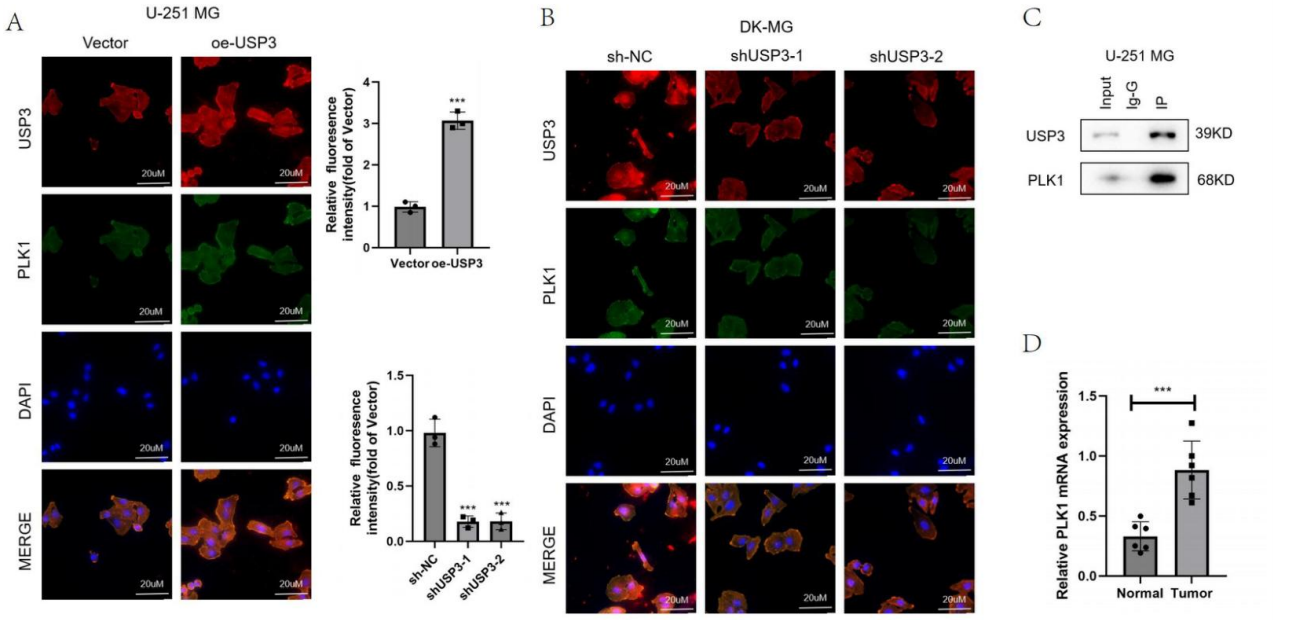

Figure 4. USP3 binds to PLK1. (A) Immunofluorescence was used to assess PLK1 expression and USP3–PLK1 colocalization in U-251MG cells transfected with a USP3 overexpression plasmid. **(B)** Immunofluorescence was used to assess PLK1 expression and USP3–PLK1 colocalization in DK-MG cells transfected with USP3 shRNA. **(C)** Co-immunoprecipitation was used to assess the interaction between USP3 and PLK1. **(D)** RT-qPCR showed higher PLK1 mRNA levels in glioma tissues than in normal tissues (Mann–Whitney U test). *P < 0.05, **P < 0.01, ***P < 0.001.

USP3 regulates PLK1 protein stability

To identify candidate USP3-regulated proteins, UbiBrowser was used for interaction prediction (Fig. 3A). PLK1, a key cell cycle regulator, was predicted to be associated with USP3. TIMER analysis indicated elevated PLK1 expression across multiple tumor types (Fig. 3B). GEPIA2 analysis indicated elevated PLK1 expression in glioma (Fig. 3C). Correlation analysis showed a positive association between USP3 and PLK1 expression (Fig. 3D). PLK1 protein levels were then assessed after USP3 modulation. PLK1 protein levels increased in USP3-overexpressing U-251MG cells (Fig. 3E). In DK-MG cells with USP3 shRNA, USP3 and PLK1 protein levels were reduced (Fig. 3G). In contrast, RT-qPCR showed no significant differences in PLK1 mRNA levels across treatment groups (Fig. 3F and Fig. 3H). These results supported post-transcriptional regulation of PLK1 by USP3.

USP3 and PLK1 interact

Immunofluorescence analysis showed increased PLK1 signal in USP3-overexpressing U-251MG cells (Fig. 4A) and decreased PLK1 signal in DK-MG cells with USP3 shRNA (Fig. 4B). Co-immunoprecipitation assays supported an interaction between USP3 and PLK1 (Fig. 4C). RT-qPCR analysis of clinical tissues showed higher PLK1 mRNA levels in glioma tissues than in normal tissues (Fig. 4D), which aligned with the observed elevation of USP3 in glioma samples.

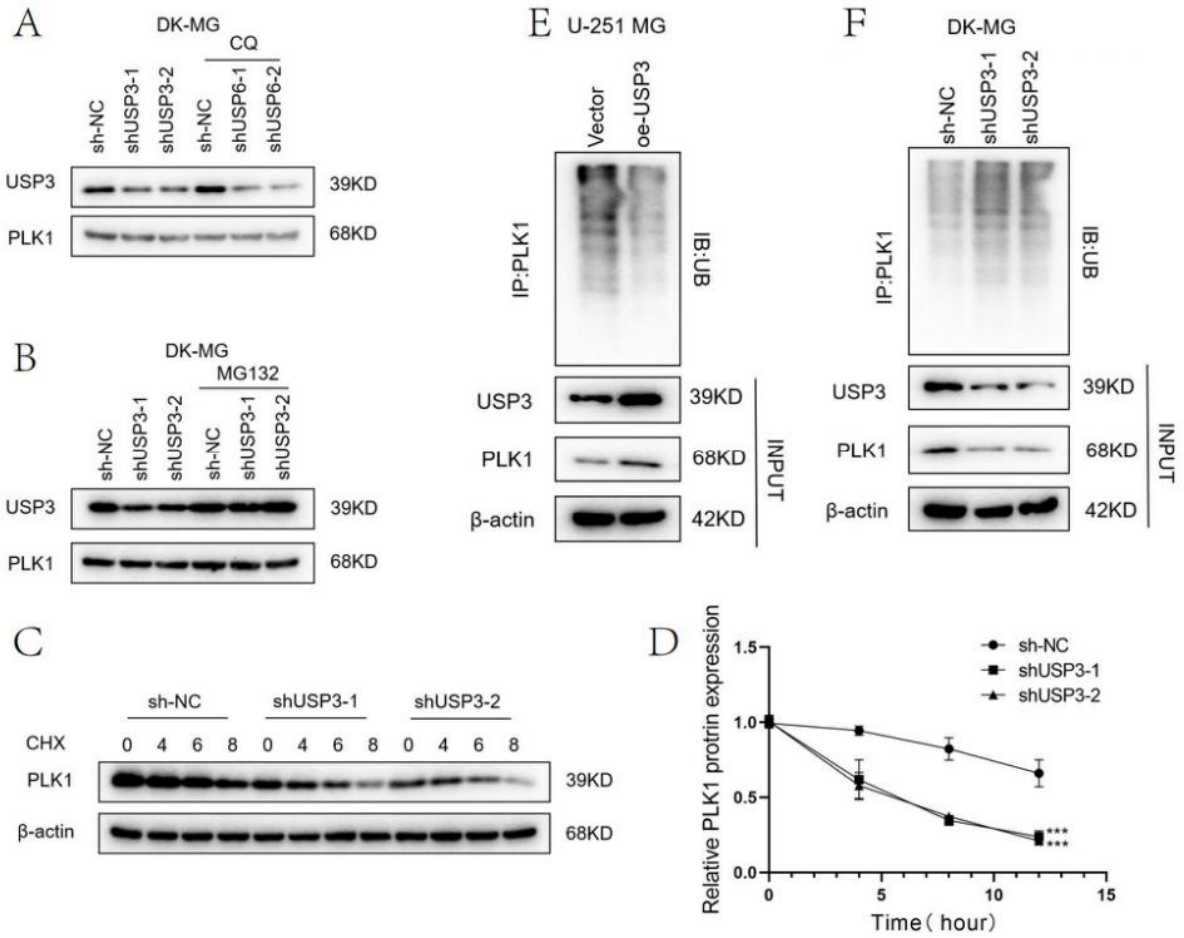

Figure 5. USP3 inhibits PLK1 degradation through the ubiquitin-proteasome pathway. (A, B) Western blot analysis of PLK1 protein levels in DK-MG cells transfected with USP3 shRNA and treated with chloroquine (200 μM, 8 h) (A) or MG132 (10 μM, 8 h) (B). **(C, D)** Cycloheximide (CHX) chase assays showed accelerated PLK1 degradation in USP3-depleted DK-MG cells (C), with quantification indicating reduced PLK1 protein stability (D). **(E, F)** Ubiquitination assays showed reduced PLK1 ubiquitination in USP3-overexpressing U-251MG cells (E) and increased PLK1 ubiquitination in USP3-depleted DK-MG cells (F), supporting USP3-mediated stabilization of PLK1 via the ubiquitin-proteasome system (Mann–Whitney U test). *P < 0.05, **P < 0.01, ***P < 0.001.

USP3 regulates PLK1 through the ubiquitin-proteasome pathway

Mechanistic studies were performed to define how USP3 influences PLK1 protein levels. DK-MG cells transduced with USP3 shRNA were treated with MG132 (10 μM) or chloroquine (200 μM) for 8 h. MG132 partially restored PLK1 protein levels in USP3-silenced cells, while chloroquine did not (Fig. 5A and Fig. 5B). These findings supported proteasome-dependent degradation of PLK1. Protein stability was then assessed using cycloheximide (CHX, 10 mg/mL) for 0 h, 4 h, 6 h, and 8 h. CHX treatment reduced PLK1 protein levels over time, and PLK1 degradation was faster in USP3-silenced cells (Fig. 5C and Fig. 5D). Ubiquitination assays showed reduced PLK1 ubiquitination after USP3 overexpression (Fig. 5E) and increased PLK1 ubiquitination after USP3 silencing (Fig. 5F). Together, these data supported a model in which USP3 stabilizes PLK1 by reducing its ubiquitination and proteasomal degradation.

USP3 promotes glioma growth through PLK1 in vivo An in vivo xenograft model was used to assess the role of the USP3 and PLK1 axis. Male nude mice aged 4 to 6 weeks were implanted subcutaneously with 2×10^6 DK-MG cells with stable USP3 silencing or matched control cells. Tumor growth was monitored after tumors reached approximately 2 mm in diameter. Tumor volume was reduced in the USP3-silenced group relative to controls, supporting a growth-promoting role for USP3. PLK1 overexpression in USP3-silenced cells reversed the

reduction in tumor growth (Fig. 6A to Fig. 6C). Immunohistochemistry showed reduced USP3 staining in the USP3-silenced group, along with reduced Ki-67 and PLK1 staining. In the PLK1 rescue group, USP3 staining remained low, while Ki-67 and PLK1 staining increased (Fig. 6D and Fig. 6E). These results supported PLK1 as a functional downstream effector of USP3 in glioma growth.

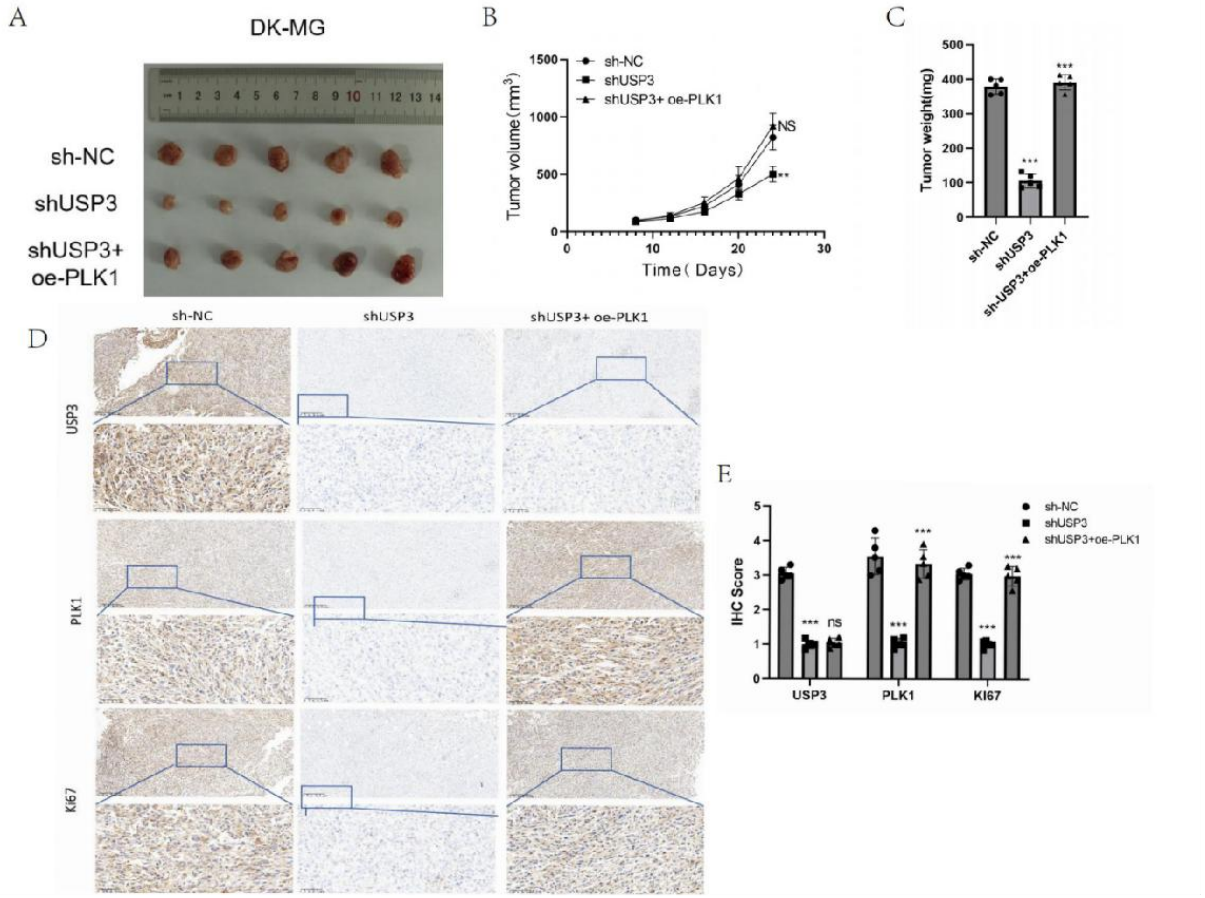

Figure 6. USP3 promotes glioma xenograft growth through PLK1 *in vivo*. (A) DK-MG cells were transduced with USP3 shRNA and then transfected with a PLK1 overexpression plasmid before implantation. **(B)** Tumor weight at endpoint. **(C)** Tumor volume was measured every 5 days starting when tumors reached 2 mm in diameter. **(D)** Immunohistochemistry was performed to assess USP3, PLK1, and Ki-67 expression in xenograft tissues. **(E)** Quantification of immunohistochemistry (Mann–Whitney U test). *P < 0.05, **P < 0.01, ***P < 0.001.

DISCUSSION

This study identified elevated USP3 expression in glioma tissues and linked USP3 to proliferative and invasive phenotypes in glioma cells. Functional assays showed that USP3 overexpression increased proliferation, migration, and invasion, while USP3 silencing reduced these phenotypes. Mechanistic studies supported PLK1 as a downstream target regulated at the protein level. PLK1 protein levels tracked with USP3 modulation, while PLK1 mRNA levels remained unchanged, which supported post-transcriptional control.

Prior work has linked USP3 dysregulation to malignant behavior in several tumor types, including breast cancer and lung cancer [13]. PLK1 overexpression has also been reported in multiple cancers and has been associated with rapid proliferation and poor prognosis [14, 15]. In colorectal cancer, PLK1 overexpression accelerates cell cycle progression and promotes tumor growth [16]. USP3 has been implicated in growth and survival pathways in other contexts, including osteosarcoma through EPHA2 and PI3K and AKT signaling, liver cancer drug resistance, and prostate cancer DNA damage response through SMARCA5 stabilization [17-19]. These studies support a broader oncogenic role for USP3 and PLK1 across tumor settings.

PLK1 functions as a central regulator of mitosis and cell cycle progression [20]. PLK1 stability and activity are controlled by post-translational modifications, including ubiquitination [21]. PLK1 degradation occurs primarily through the ubiquitin-proteasome system, with multiple E3 ligases contributing to PLK1 ubiquitination and

turnover [22, 23]. The present data supported direct interaction between USP3 and PLK1 and showed that USP3 reduced PLK1 ubiquitination. MG132 rescue, CHX chase kinetics, and ubiquitination assays together supported proteasome-dependent regulation.

Reports outside oncology also link USP3 to protein homeostasis. PM2.5 exposure has been reported to reduce USP3 activity, accelerate SIRT3 degradation, and increase p53 acetylation, which promotes senescence and ferroptosis in type II alveolar epithelial cells [24]. In osteoarthritis chondrocytes, USP3 stabilizes SIRT3, promotes FOXO3 deacetylation, and suppresses IL-1β-induced ROS and cell cycle arrest, which reduces senescence [25, 26]. In reproductive aging studies, maternal aging has been linked to reduced expression of components of the dynein-dynactin-PLK1 axis, which associates with meiotic defects and reduced oocyte competence [27, 28]. These findings support broader roles for USP3 and PLK1 in stress responses and aging-related processes.

Overall, the data supported a USP3 to PLK1 regulatory axis in glioma. USP3 reduced PLK1 ubiquitination, stabilized PLK1 protein, and promoted malignant phenotypes. Future work should define the relevant E3 ligases and map the molecular determinants of the USP3 and PLK1 interaction in glioma.

Conclusion

USP3 stabilized PLK1 by deubiquitination and promoted glioma cell proliferation, migration, and invasion. The USP3 and PLK1 axis represents a potential therapeutic target in glioma. Disrupting the USP3 and PLK1 interaction is expected to suppress glioma growth and progression. Further studies should define the E3 ligases that cooperate with this pathway and support development of targeted interventions.

Author contributions

Feng Yan: project development, experimental design, data analysis, manuscript writing. Yongzhi Shan: project development, experimental design. Xiaotong Fan: experimental execution. Yaming Wang: data collection, data analysis. Penghu Wei: experimental execution. All authors reviewed the manuscript and approved the submitted version.

Acknowledgments

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Conflict of interest

The authors have declared that no conflict of interest exists.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supplementary Materials

The Supplementary data can be found online at: www.aginganddisease.org/EN/10.14336/AD.2025.01011.

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